media is replaced with media containing penicillin/streptomycin at standard tissue culture concentrations. The cells were then incubated in the presence or absence of 100nM atRA for 72 hours. After incubation, cells are washed twice with phosphate-buffered saline and collected in 100 μL of Reporter Lysis Buffer (Promega). The lysate is left at room temperature for 10 minutes followed by 1 cycle of freeze/thaw using a dry ice-ethanol bath for freezing the cell sample and thawing in a 37°C water bath. 50μL aliquots are transferred to fresh tubes for Firefly Luciferase Assay (Promega). Luciferase activity is measured as described above using a Turner 20/20 luminometer at 47.9% sensitivity with a 5 sec. delay period and 15 sec. integration time. An additional aliquot is removed from the cell lysate to measure protein concentration using Bio-Rad protein assay kit (Bradford assay). Luciferase activity for each sample is normalized to protein content and expressed as luciferase/μg protein. All assays are carried out in triplicate and displayed as a mean and standard deviation.

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REMARKS

1. Status of the Claims

Claims 1-20 were pending at the issuance of the instant Office Action. Claims 1-7, 19, and 20 are cancelled without prejudice or disclaimer pursuant to Applicants' election of the claims of Group II in the restriction requirement asserted in this application. Claims 8, 13, and 17 have been amended. The amendments to the claims are fully supported by the specification. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

2. Specification

The Action asserts that the only definition of a RARE site found in the specification is at page 12 and 13, and is in reference to a publication. The Action points out that incorporation of essential material in the specification by reference to a publication is improper. Applicants have amended the specification to include the definition of RARE sites as found in the publication. A declaration is enclosed to confirm that the amendment does not introduce new matter into the specification.

In addition, the disclosure was objected to because it contained an embedded hyperlink at page 26, line 22. The specification has been amended, thereby obviating this objection.

The disclosure was also objected to because it contained blank lines at numerous places within the specification. The specification has been amended to overcome this objection.

Claims 8-11 were objected to as depending upon a nonelected claim. The claims have been amended to overcome this objection.

3. The claims fulfill all the requirements of 35 U.S.C. §112

Claim 13 stands rejected under 35 U.S.C. §112, second paragraph as being indefinite because it refers to NCBI accession numbers rather than sequences found in the specification, and because it refers to itself. Claim 13 has been amended to refer to SEQ ID numbers rather than accession numbers. The specification provides SEQ ID numbers for all of the genes referred to in claim 13 (see page 5, lines 12-22; page 6, lines 8-19; page 7, lines 13-23; and page 15, line 22 to page 16, line 16). Therefore, although the genes are also referred to by Accession Numbers elsewhere in the specification, the genes are not incorporated by reference. Rather, the genes are properly referred to by SEQ ID numbers and can be found in the Sequence Listing. In addition, claim 13 has been amended to properly refer to claim 12 rather than to itself. Consequently, Applicants submit that claim 13 as amended satisfies the statutory requirements of 35 U.S.C. §112, first paragraph, and respectfully request that this ground of rejection be withdrawn.

Claim 17 stands rejected under 35 U.S.C. §112, first paragraph as being unclear as to the metes and bounds of the phrase "assaying for an activity of the cellular gene product." Claim 17 has been amended to specifically recite that the cellular genes whose products are being assayed are the cellular genes recited in claim 13. Applicants submit that the amendment to claim 17 clarifies that "assaying for an activity of a cellular gene product" means assaying for an activity of a cellular gene product of a specified gene, that is a gene also identified in claim 13. Withdrawal of this rejection is, therefore, respectfully solicited.

Claims 8-16, and 18 stand rejected under 35 U.S.C. §112, first paragraph, as being enabled for MCF-7 cells only. The Action asserts that undue experimentation would be required to enable the full scope of the claims, which encompasses assaying for changes in expression of retinoid-induced cellular genes in a mammalian cell. The Action asserts that, while the specification teaches that all the cellular genes listed in claim 13 are induced by retinoic acid in MCF7 cells, the

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specification does not teach that the genes are induced by retinoic acid in *any other* mammalian cell. Applicants respectfully traverse.

Applicants point out that the specification teaches and claims methods for identifying compounds that induce retinoid-induced cellular genes. As disclosed in Applicants' specification, such compounds are *not* retinoids, which are known to induce said genes in cells such as MCF-7. The invention provides methods for identifying compounds that induce expression of retinoid-inducible genes, but which compounds are not retinoids and do not require retinoid receptors for their action. Although the genes must be inducible by retinoids in certain cell lines, the genes are not necessarily inducible by retinoids in the cell line used in a screening method of the invention. Thus, methods of the invention can be used to identify compounds that induce retinoid-induced genes in cells that are totally unresponsive to retinoids (*e.g.* cells that lack retinoid receptors). Consequently, Applicants contend that the claims are enabled for *any* mammalian cell, because said cells do not need to be sensitive to retinoids or express the retinoid receptor; the cells must simply induce expression of retinoid-inducible genes in response to the presence of the test compound. Applicants thus respectfully contend that their argument has traversed the asserted ground of rejection, and request that this ground of rejection be withdrawn.

Claim 17 stands rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not adequately described in the specification. Specifically, the Action asserts that the metes and bounds for assaying an activity of the cellular gene product are not clear. Applicants respectfully traverse.

Claim 17 as amended encompasses an assay for an activity of the gene product of a cellular gene that is recited in claim 13. The genes in claim 13 are known genes having known activities, as described in the specification (see Example 2). If the activity of a gene is known, one of skill in the art can readily choose an assay to detect that activity without undue experimentation. The skilled worker will recognize how to measure the results, establish controls, and various other parameters of such assays using conventional methods known in the art. Thus, Applicants contend that the invention is adequately described based on the teaching in the specification combined with conventional methods known to the skilled worker. Therefore, Applicants respectfully request that this ground of rejection be withdrawn.

Claim 17 also stands rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that is not enabled by the specification. Specifically, the Action asserts that the assay, to

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which claim 17 is drawn, is not taught in the specification. As discussed in the preceding paragraph, claim 17 has been amended to recite the genes in claim 13. Also, an assay for detecting an activity of those genes can be readily designed by one of skill in the art using the teaching of the specification and known assay methods. Consequently, Applicants submit that this rejection has been overcome by amendment. Reconsideration and withdrawal of this ground of rejection is kindly solicited.

4. The claims are patentable under 35 U.S.C. §103

Claims 8-16, and 18 stand rejected under 35 U.S.C. §103 as being unpatentable over Adamo *et al.*, Miller, Han *et al.*, and US Pat. 5,795,726 (the '726 patent).

The Action points out that Adamo *et al.* teach IGFBP-3 expression in MCF-7 cells and Han *et al.* teach that IGFBP-3 is induced by retinoids, but neither discuss the presence or absence of a RARE site in its promoter. Furthermore, the Action indicates that Miller teaches that retinoids and retinoid analogs may be useful for treating cancer, and that the '726 patent teaches methods for identifying compounds that modulate human hepatic nuclear factor-1 (HNF-1) activity.

The references cited in the Action, alone or in combination, do not teach the claimed invention. The Action concedes that Adamo *et al.* and Han *et al.* do not teach the method claimed in claim 12, for identifying a non-retinoid compound that induces expression of a retinoid-induced gene. However, the Action asserts that Miller teaches the need for research to develop alternative novel retinoid compounds for clinical use, citing page 1473, the last 5 lines of paragraph 2 and pages 1475-1467. Miller, however, does not teach identification of *non*-retinoid compounds using a promoter from a retinoid-induced gene that lacks a RARE site, nor does Miller teach any other method of identifying such compounds. In addition, Miller does not suggest that using a promoter that does *not* contain a RARE site from IGFBP-3 or any other gene whose expression is modulated by retinoids, or that such promoter constructs are useful in assays to identify such compounds. The '726 patent does not provide any teaching related to retinoid-induced genes that lack RARE sites. Rather, the '726 patent is specifically drawn to methods for identifying compounds that modulate HNF-1 activity useful for treating Type II diabetes. Indeed, the '726 patent contains no teachings of compounds that specifically induce expression of retinoid-induced genes that lack a RARE site.

Miller provides only generalized teaching regarding the potential application of retinoids and retinoid analogs in cancer therapies. Miller's precatory statements regarding retinoids and retinoid analogs and cancer therapeutics combined with the teachings of the other references cited in the

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Action do not provide specific motivation to use genes encompassed by the Applicants' invention to identify non-retinoid compounds that induce expression of retinoid-induced genes. It is only in Applicants' specification that motivation is provided to the skilled worker to use genes that do not contain a RARE site for detecting non-retinoid compounds that induce expression of retinoid-induced genes.

Applicants, therefore, submit that these references when taken separately or in combination do not render the instant invention obvious, and respectfully request this rejection be withdrawn.

CONCLUSIONS

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

The Examiner is invited to contact the undersigned representative by telephone at (312) 913-0001 if it is believed to be helpful.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Date: November 18, 2002

By: _____
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RED-LINED VERSION OF THE AMENDED CLAIMS

99-216-H (Application No. 09/865,879)

8. (Amended) A method for identifying a compound that induces expression of a retinoid-inducible gene in a mammalian cell, the method comprising the steps of:

(a) culturing a recombinant mammalian cell in the presence and absence of a compound, wherein the recombinant mammalian cell comprises a recombinant expression construct encoding a reporter gene operably linked to a promoter from a gene the expression of which is induced by a retinoid, wherein the promoter does not contain a RARE site;

(b) comparing reporter gene expression in said cell in the presence of the compound with reporter gene expression in said cell in the absence of the compound; and

(c) identifying the compound that induces retinoid-induced gene expression if reporter gene expression is higher in the presence of the compound than in the absence of the compound.

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13. (Amended) The method of claim 12, wherein the cellular gene is insulin-like growth factor binding protein-3 (IGFBP-3; SEQ ID NO: 1), secreted cell adhesion protein βIG-H3 (SEQ ID NO: 2), epithelial protein lost in neoplasm (EPLIN; SEQ ID NO: 3), ubiquitin-like protein FAT10 (SEQ ID NO: 4), Mac-2 binding protein (Mac-2 BP; SEQ ID NO: 6), Protein C inhibitor (PCI; SEQ ID NO: 7), T cell receptor gamma (SEQ ID NO: 8), retinal oxidase (SEQ ID NO: 9), Bene (SEQ ID NO: 10), HIF-2alpha/EPAS-1 (SEQ ID NO: 11), selectin L (SEQ ID NO: 12), or proteasome activator PA28 subunit α (PA28α; SEQ ID NO: 5).

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17. (Amended) The method of claim 12, wherein expression of the cellular gene is detected by assaying for an activity of the cellular gene product, wherein the cellular gene is insulin-like growth factor binding protein-3 (IGFBP-3, SEQ ID NO: 1), secreted cell adhesion protein βIG-H3 (SEQ ID NO: 2), epithelial protein lost in neoplasm (EPLIN; SEQ ID NO: 3), ubiquitin-like protein FAT10 (SEQ ID NO: 4), Mac-2 binding protein

(Mac-2 BP; SEQ ID NO: 6), Protein C inhibitor (PCI; SEQ ID NO: 7), T cell receptor gamma (SEQ ID NO: 8), retinal oxidase (SEQ ID NO: 9), Bene (SEQ ID NO: 10), HIF-2alpha/EPAS-1 (SEQ ID NO: 11), selectin L (SEQ ID NO: 12), or proteasome activator PA28 subunit α (PA28α; SEQ ID NO: 5).

RED-LINED VERSION OF THE REPLACEMENT PARAGRAPHS

99-216-H (Application No. 09/865,879)

Page 2, line 5-17:

The target of retinoid action is the cell nucleus, where retinoids bind to two types of receptors, termed RARs (retinoic acid receptors) and RXRs (retinoid X receptors) (Mangelsdorf *et al.*, 1994, "The retinoid receptors," *in:* The Retinoids: biology, chemistry, and medicine, Sporn *et al.*, eds., New York: Raven Press, pp. 319-351.) Retinoid-bound receptor molecules form homo- (RXR-RXR) and heterodimers (RAR-RXR) that act as transcription factors. These dimers bind to specific *cis*-regulatory sequences in the promoters of retinoid-responsive target genes, termed RARE (Retinoic Acid Response Elements), regulating their transcription.

6.19
19.1

A RARE sequence has a minimal half-site consensus sequence that is generally well conserved as AGGTCA or AGTTCA (Mangelsdorf *et al.*, 1994, "The retinoid receptors," *in*: The Retinoids: biology, chemistry, and medicine, Sporn *et al.*, eds., New York: Raven Press, pp. 327-331). RAREs are typically configured into one of three structured motifs: direct repeats, palindromes, and complex elements without an obvious consensus structure. The direct repeat requires a lesser amount of receptor to activate a retinoid-inducible gene than the other configurations. In addition, direct repeats separated by 5 nucleotides have demonstrated the strongest responses while moderate responses are typically generated by direct repeats separated by 2 nucleotides. (See Mangelsdorf *et al.*, 1994, "The retinoid receptors," *in*: The Retinoids: biology, chemistry, and medicine, Sporn *et al.*, eds., New York: Raven Press, pp. 327-331.)

The resulting changes in gene expression are caused either directly by retinoid receptor regulation of target gene expression, or indirectly through the action of retinoid-activated signal transduction pathways, *for example*, pathways activated by the transcription factor AP-1. These gene expression changes are ultimately responsible for the growth-inhibitory effect of retinoids (Warrell, *Id.*).

Page 11, line 24 to page 12, line19:

Another advantage of such compounds is that they can be expected to have a growth-inhibitory effect without producing systemic side effects found with other growth-inhibitory compounds known in the prior art. For example, many growth-inhibitory drugs and compounds known in the prior art disadvantageously induce p21 gene expression, which induces senescence, growth arrest and apoptosis by activating a plurality of genes, the expression of which is associated with the development of diseases, particularly age-related diseases such as Alzheimer's disease, atherosclerosis, renal disease, and arthritis (as disclosed in co-owned and co-pending U.S. Serial No. 60/265,840, filed February 1, 2001, and U.S. Serial No. 09/861,925, filed May 21, 2001, incorporated by reference herein, Retinoic acid-induced growth inhibition in MCF-7 cells, in contrast, does not induce p21 (Zhu *et al.*, 1997, *Exp. Cell Res.* 234:293-299). The genes identified herein that are induced by retinoids are not known to be associated with any disease or disadvantageous or pathogenic effect when expressed in an animal. Thus, identification of such compounds that mimic the growth-inhibitory effects of retinoids by inducing expression of one or a plurality of the genes identified herein can be expected to have reduced or no such side-effects, making them better agents for anti-tumor and other therapies. Discovery of compounds that mimic the growth-inhibitory effects of retinoids without producing the toxic side effects of growth-inhibitory compounds known in the art is thus advantageously provided by the invention.

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Page 26, lines 18-22:

These sequences were then analyzed for the presence of two closely spaced hexameric core motifs of RARE sites (Mangelsdorf *et al.*, 1994, in THE RETINOIDS: BIOLOGY, CHEMISTRY, AND MEDICINE, (Sporn *et al.*, eds.), pp. 327-330 (Raven Press, New York), in variable orientations, using the "Regulatory Sequence Analysis Tools" available from the University of Brussels, Belgium,

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Page 28, line 10, to page 29, line 11:

The ability of this construct to drive retinoid-inducible luciferase expression in mammalian cells is demonstrated in transient transfection assay, as described in U.S. Provisional Patent Application Serial No. 60/265,840, filed February 1, 2001 and U.S. Patent Application Serial No. 09/861,925, filed May 21, 2001, incorporated by reference herein. Briefly, transfection is carried out using LIPOFECTAMINE 2000 (Life Technologies, Inc. Gaithersburg). Cells are plated at a density of 70,000 cells/well in 12 well plates in 1 mL, media containing 2mM glutamine, 10% FBS, 0.1mM NEAA (Non-Essential Amino Acids, GIBCO), 1mM sodium pyruvate, and 10 μg/mL insulin, and without penicillin/streptomycin. After culturing the cells for a sufficient time that they attached to the culture dish, transfection was performed in triplicate according to the manufacturer's instructions, using 1 μg pGL2- basic vector DNA and 1 μg pGL2-βIG-H3 promoter DNA. After 10 hours, culture media is replaced with media containing penicillin/streptomycin at standard tissue culture concentrations. The cells were then incubated in the presence or absence of 100nM atRA for 72 hours. After incubation, cells are washed twice with phosphate-buffered saline and collected in 100 μL of Reporter Lysis Buffer (Promega). The lysate is left at room temperature for 10 minutes followed by 1 cycle of freeze/thaw using a dry ice-ethanol bath for freezing the cell sample and thawing in a 37°C water bath. 50μL aliquots are transferred to fresh tubes for Firefly Luciferase Assay (Promega). Luciferase activity is measured as described above using a Turner 20/20 luminometer at 47.9% sensitivity with a 5 sec. delay period and 15 sec. integration time. An additional aliquot is removed from the cell lysate to measure protein concentration using Bio-Rad protein assay kit (Bradford assay). Luciferase activity for each sample is normalized to protein content and expressed as luciferase/μg protein. All assays are carried out in triplicate and displayed as a mean and standard deviation.

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